

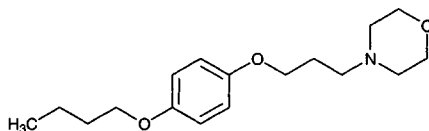
**SAMPLE****Matrix:** formulations**Sample preparation:** Condition a 6 mL 200 mg C4 SPE cartridge (Baker) with MeCN and water. Add the liquid formulation to the SPE cartridge, wash with water, elute with MeCN:water 40:60 containing 0.1% trifluoroacetic acid. Evaporate the eluate to dryness and dissolve the residue in 30 mM pH 4.0 sodium acetate so as to obtain a 2 mg/mL solution. Inject an aliquot.**HPLC VARIABLES****Column:** 250 × 4.6 5 µm Symmetry C8**Mobile phase:** Gradient. A was MeCN:200 mM potassium dihydrogen phosphate 6.05:93.95, pH 3.0. B was MeCN:200 mM potassium dihydrogen phosphate 22.45:77.55, pH 3.0. C was MeCN:200 mM potassium dihydrogen phosphate 26.9:73.1, pH 3.0. A:B:C from 100:0:0 to 0:100:0 over 16 min, maintain at 0:100:0 for 69 min, to 0:0:100 over 15 min, maintain at 0:0:100 for 10 min**Column temperature:** 55**Flow rate:** 0.5**Detector:** UV 220**CHROMATOGRAM****Retention time:** 57**OTHER SUBSTANCES****Extracted:** degradation products**KEY WORDS**

liquid formulation; SPE

**REFERENCE**

Hekman,C.M.; DeMond,W.; Dixit,T.; Mauch,S.; Nuechterlein,M.; Stepanenko,A.; Williams,J.D.; Ye,M. Isolation and identification of peptide degradation products of heat stressed pramlintide injection drug product, *Pharm.Res.*, **1998**, *15*, 650–659.

# Pramoxine

**Molecular formula:** C<sub>17</sub>H<sub>27</sub>NO<sub>3</sub>**Molecular weight:** 293.41**CAS Registry No.:** 140-65-8, 637-58-1 (HCl)**Merck Index:** 7888**Lednicer No.:** 1 18**SAMPLE****Matrix:** formulations**Sample preparation:** Collect contents of an aerosol can using 100 mL ether:MeOH 5:1, extract with 100 mL 20% acetic acid, extract with 75 mL 20% acetic acid, combine the extracts, make up to 250 mL with water, inject a 15 µL aliquot.**HPLC VARIABLES****Column:** 250 × 4 10 µm µBondapak C18**Mobile phase:** MeOH:water:acetic acid:methanesulfonic acid 50:48.9:1:0.1**Flow rate:** 2**Injection volume:** 15**Detector:** UV 286**CHROMATOGRAM****Retention time:** 5**OTHER SUBSTANCES****Simultaneous:** methyl paraben

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**KEY WORDS**aerosol

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**REFERENCE**

Weinberger,R.; Mann,B.; Posluszny,J. High-pressure liquid chromatographic analysis of pramoxine hydrochloride in high lipid aerosol foam dosage form, *J.Pharm.Sci.*, **1980**, 69, 475–477.

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**SAMPLE**

**Matrix:** formulations

**Sample preparation:** Weigh out cream or suppository containing 18 mg pramoxine hydrochloride, add 15 mL isopropanol, add 40 mL MeOH, warm until the sample dissolves, add 40 mL MeOH, add 5 mL 4  $\mu$ L/mL dibutyl phthalate in MeOH, cool to 10° or lower, filter (0.4  $\mu$ m polycarbonate), inject an aliquot.

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**HPLC VARIABLES**

**Guard column:** 30  $\times$  4.6 C18 (Brownlee)

**Column:** 300  $\times$  3.9  $\mu$ Bondapak C18

**Mobile phase:** MeCN:10 mM pH 7.5 K<sub>2</sub>HPO<sub>4</sub> buffer 11:10 (Use a 150  $\times$  4 column of 32-63  $\mu$ m silica (ICN) between pump and injector.)

**Flow rate:** 2

**Injection volume:** 20

**Detector:** UV 224

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**CHROMATOGRAM**

**Retention time:** 12

**Internal standard:** dibutyl phthalate

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**KEY WORDS**

cream; suppositories; stability-indicating

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**REFERENCE**

Chang,Z.L.; Boller,J.P.; Pacenti,D.M.; Wong,C.F. Rapid high-performance liquid chromatographic determination of pramoxine hydrochloride in topical cream and suppositories, *J.Chromatogr.*, **1984**, 291, 428–433.

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**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Inject a 5  $\mu$ L aliquot.

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**HPLC VARIABLES**

**Column:** 300  $\times$  4 10  $\mu$ m  $\mu$ Bondapak C18

**Mobile phase:** MeCN:MeOH:water 20:20:60 containing 0.06% sulfuric acid, 0.5% sodium sulfate, and 0.02% sodium heptanesulfonate, pH 2.6

**Flow rate:** 2

**Injection volume:** 5

**Detector:** UV 305

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**CHROMATOGRAM**

**Retention time:** 8

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**OTHER SUBSTANCES**

**Simultaneous:** benzocaine, butamben, lidocaine, procaine, tetracaine

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**REFERENCE**

Menon,G.N.; Norris,B.J. Simultaneous determination of tetracaine and its degradation product, p-n-butylaminobenzoic acid, by high-performance liquid chromatography, *J.Pharm.Sci.*, **1981**, 70, 569–570.

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**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Prepare a 10  $\mu$ g/mL solution in MeOH, inject a 20  $\mu$ L aliquot.

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**HPLC VARIABLES**

**Column:** 125 × 4.9 Spherisorb S5W silica

**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

**Flow rate:** 2

**Injection volume:** 20

**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

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**CHROMATOGRAM**

**Retention time:** 1.4

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**OTHER SUBSTANCES**

**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazepine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipiprone, diprenorphine, dipyrindamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclorphenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozone, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranlycypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleminamine, triprolidine, tryptamine, verapamil, xylometazoline

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**REFERENCE**

Jane, I.; McKinnon, A.; Flanagan, R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J. Chromatogr.*, **1985**, *323*, 191–225.

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**SAMPLE**

**Matrix:** solutions

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**HPLC VARIABLES**

**Column:** 250 × 4.6 5 µm Supelcosil LC-DP (A) or 250 × 4.5 µm LiChrospher 100 RP-8 (B)

**Mobile phase:** MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)

**Flow rate:** 0.6

Injection volume: 25

Detector: UV 229

**CHROMATOGRAM**

Retention time: 11.16 (A), 6.25 (B)

**OTHER SUBSTANCES**

**Also analyzed:** acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordi-azepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxy-chloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazin-dol, mefenamic acid, meperidine, mephénytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyl dopa, methylphenidate, metoclopramide, metolazone, metoprolol, met-ronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymet-azoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, phen-iramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltolox-amine, phenytoin, pimozide, pindolol, piroxicam, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propranthe line, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, qui-nine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, seco-barbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocanide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazo-line, yohimbine, zopiclone

**KEY WORDS**

details of plasma extraction

**REFERENCE**

Koves, E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J. Chromatogr. A*, **1995**, 692, 103–119.

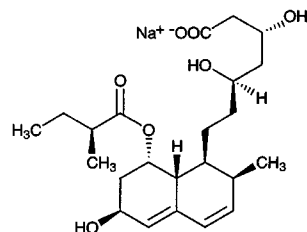
# Pravastatin sodium

Molecular formula:  $C_{23}H_{35}NaO_7$ 

Molecular weight: 446.52

CAS Registry No.: 81131-70-6, 81093-37-0 (free acid)

Merck Index: 7894

**SAMPLE****Matrix:** blood, feces, urine

**Sample preparation:** Plasma. 2 mL Plasma + 4 mL MeCN, centrifuge at 700 g for 10 min, remove the supernatant and wash the precipitate twice with 2 mL MeCN:water 2:1. Combine the supernatants and evaporate them to dryness under vacuum, reconstitute the residue in 1 mL MeCN:water 2:1, centrifuge at 10000 g, remove the supernatant and add it to 500  $\mu$ L

water, centrifuge at 10000 g, inject a 1 mL aliquot. Urine. Centrifuge at 10000 g, inject an aliquot. Feces. Homogenize feces, 1 g homogenate + 2 mL MeCN, sonicate for 5 min, shake in a wrist action shaker for 20 min, centrifuge at 700 g for 10 min. Remove the supernatant and wash the precipitate twice with 1 mL MeCN:water 2:1, combine the supernatants, inject a 500  $\mu$ L aliquot.

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**HPLC VARIABLES**

**Guard column:** present but not specified

**Column:** 500  $\times$  9.4 Partisil 10 ODS-3 C18

**Mobile phase:** Gradient. MeCN:10 mM pH 7.2 potassium phosphate buffer containing 5 mM tetrabutylammonium hydrogen sulphate at 25:75 for 20 min, then to 50:50 over 45 min, hold at 50:50 for 10 min

**Flow rate:** 4

**Injection volume:** 500-1000

**Detector:** Collect fractions and measure radioactivity (UV maximum absorbance at 245 nm)

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**CHROMATOGRAM**

**Retention time:** 33

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**OTHER SUBSTANCES**

**Extracted:** metabolites

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**KEY WORDS**

plasma; semi-preparative; radiolabeled starting material

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**REFERENCE**

Everett,D.W.; Chando,T.J.; Didonato,G.C.; Singhvi,S.M.; Pan,H.Y.; Weinstein,S.H. Biotransformation of pravastatin sodium in humans, *Drug Metab.Dispos.*, **1991**, 19, 740-748.

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**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Centrifuge at 2000 rpm, inject an aliquot.

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**HPLC VARIABLES**

**Column:** 300  $\times$  3.9  $\mu$ Bondapak

**Mobile phase:** MeOH:water:triethylamine:glacial acetic acid 500:500:1:1

**Column temperature:** 30

**Flow rate:** 1.3

**Detector:** UV 238

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**CHROMATOGRAM**

**Retention time:** 17.5 (10.1 hydroxy acid form)

**Limit of detection:** 10 ng/mL

**Limit of quantitation:** 25 ng/mL

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**REFERENCE**

Serajuddin,A.T.; Ranadive,S.A.; Mahoney,E.M. Relative lipophilicities, solubilities, and structure-pharmacological considerations of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors pravastatin, lovastatin, mevastatin, and simvastatin, *J.Pharm.Sci.*, **1991**, 80, 830-834.

# Prazepam

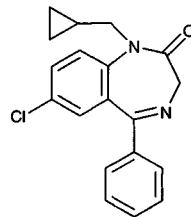
**Molecular formula:** C<sub>19</sub>H<sub>17</sub>ClN<sub>2</sub>O

**Molecular weight:** 324.81

**CAS Registry No.:** 2955-38-6

**Merck Index:** 7895

**Lednicer No.:** 2 405



## SAMPLE

**Matrix:** bile, blood, gastric contents, tissue, urine

**Sample preparation:** Chop 5-g tissue and homogenize (Ultra Turrax T25) at 8500, 9500, 13500, 20500, and 24000 rpm for 1 min each. Add homogenate to 20 mL water. Dilute blood, urine, gastric contents, and bile four times with water. Mix 4 mL sample with 4 mL diethyl ether and mix for 15 min (Spiramix 10, Denley, UK). Separate the organic layer, add 4 mL diethyl ether to extraction sample, mix. Evaporate combined organic layers to dryness under a stream of dry air at 50°. Purify extracts by partition between 1 mL MeCN and 2 mL heptane, separate MeCN layer, evaporate it to dryness, reconstitute the residue in 100 µL MeOH and inject a 20 µL aliquot of the solution.

## HPLC VARIABLES

**Guard column:** 20 × 4.6 5 µm Apex II ODS

**Column:** 150 × 4.6 5 µm Apex II ODS

**Mobile phase:** MeCN:MeOH:10 mM phosphoric acid:10 mM Na<sub>2</sub>HPO<sub>4</sub> 40:20:36:4

**Flow rate:** 1

**Injection volume:** 20

**Detector:** UV 240

## CHROMATOGRAM

**Retention time:** 14.5

**Internal standard:** prazepam

**Limit of quantitation:** 100 ng/mL

## OTHER SUBSTANCES

**Extracted:** diazepam, nitrazepam, oxazepam, temazepam

## KEY WORDS

liver; lung; muscle; urine; pericardial fluid; prazepam is IS

## REFERENCE

Pounder, D.J.; Adams, E.; Fuke, C.; Langford, A.M. Site to site variability of postmortem drug concentrations in liver and lung, *J. Forensic Sci.*, **1996**, *41*, 927–932.

## SAMPLE

**Matrix:** blood

**Sample preparation:** 200 mg Extrelut + 400 µL blood + 100 µL MeOH, mix, let dry at room temperature for 1–2 h. Add to a 30 × 4.6 stainless steel extraction column, extract with carbon dioxide:ethyl acetate 95:5 at 2 mL/min, 65°, and 300 psi. for 10 min, collect by expansion into MeOH. Dry the collected extract at 65° under nitrogen. Reconstitute the residue in 50 µL mobile phase. Inject a 20 µL aliquot. Condition ca. 10 g Extrelut in a 10 mL plastic syringe with dichloromethane. Add 250 µL 5% ammonia to the top. Mix 900 µL blood with 100 µL MeOH. Add 1 mL pH 4 phosphate buffer and 250 µL 5% ammonia solution, mix thoroughly, add to the extraction column. After 5 min elute with diethyl ether under the influence of gravity. Collect 8 mL eluate, evaporate to dryness at 65° under nitrogen. Reconstitute the residue in 180 µL mobile phase, inject a 20 µL aliquot.

## HPLC VARIABLES

**Guard column:** 20 × 4.6 5 µm Hypersil ODS

**Column:** 250 × 4.6 5 µm Hypersil ODS

**Mobile phase:** MeOH:Na<sub>2</sub>HPO<sub>4</sub> 70:30

Flow rate: 1  
Injection volume: 20  
Detector: UV 254

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**CHROMATOGRAM**

Retention time: 11.5  
Internal standard: prazepam

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**OTHER SUBSTANCES**

Extracted: temazepam  
Also analyzed: diazepam, chlordiazepoxide, nordiazepam, oxazepam

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**KEY WORDS**

SFE; SPE; prazepam is IS; whole blood

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**REFERENCE**

Scott, K.S.; Oliver, J.S. Development of a supercritical fluid extraction method for the determination of temazepam in whole blood, *J. Anal. Toxicol.*, **1997**, 21, 297–300.

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**SAMPLE**

Matrix: blood

**Sample preparation:** Condition a 100 mg Bond-Elut C2 SPE cartridge with 1 volume MeOH and 1 volume 10 mM pH 8.0 phosphate buffer. 1 mL Plasma + 100  $\mu$ L 1 M pH 8.0 potassium phosphate buffer, mix, add to the SPE cartridge, wash with 3 volumes of water, wash with 1 mL MeOH:water 30:70, wash with 1 mL water, elute with 1 mL MeOH:water 70:30, elute with 1 mL water. Evaporate the eluate to dryness, reconstitute with 200  $\mu$ L mobile phase, inject an aliquot.

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**HPLC VARIABLES**

Column: 35  $\times$  4.6 5  $\mu$ m Ultrabase C18  
Mobile phase: MeOH:water 60:40  
Flow rate: 1  
Injection volume: 20  
Detector: UV 217

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**CHROMATOGRAM**

Retention time: 8  
Internal standard: prazepam

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**OTHER SUBSTANCES**

Extracted: midazolam

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**KEY WORDS**

plasma; SPE; prazepam is IS

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**REFERENCE**

Berrueta, L.A.; Gallo, B.; Vincente, F. Rapid determination of midazolam in plasma using SPE and HPLC, *Am. Lab.*, **1993**, 25 (Dec.), 20R–20T.

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**SAMPLE**

Matrix: blood

**Sample preparation:** Inject 100–200  $\mu$ L plasma onto column A with mobile phase A and elute to waste, after 5 min backflush the contents of column A onto column B with mobile phase B, after 5 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Wash column A with MeCN:water 60:40 at 1 mL/min for 6 min then re-equilibrate with pH 7.5 buffer for 10 min.

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**HPLC VARIABLES**

Column: A 45  $\times$  4 12  $\mu$ m TSK-gel G 3 PW (Tosohass); B 75  $\times$  4.6 Ultrasphere ODS C18 3  $\mu$ m

**Mobile phase:** A 50 mM pH 7.5 phosphate buffer; B Gradient. A was MeCN. B was 65 mM  $\text{KH}_2\text{PO}_4$  + 1% diethylamine adjusted to pH 5.4 with phosphoric acid. A:B 22:78 for 5 min, to 25:75 over 10 min, to 60:40 over 15 min.

**Flow rate:** 1

**Injection volume:** 100-200

**Detector:** UV 230

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## CHROMATOGRAM

**Retention time:** 29

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## OTHER SUBSTANCES

**Extracted:** alprazolam, bromazepam, chlordiazepoxide, clobazam, clonazepam, clorazepate, clobazepam, desmethyloclobazam, desmethyldiazepam, diazepam, estazolam, flunitrazepam, lorazepam, lorazepate, lorazepam, medazepam, nitrazepam, oxazepam, temazepam, tetrazepam, tofisopam, triazolam

**Noninterfering:** carbamazepine, phenytoin, ethosuximide, phenobarbital, primidone, valproic acid

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## KEY WORDS

plasma; column-switching

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## REFERENCE

Lacroix, C.; Wojciechowski, F.; Danger, P. Monitoring of benzodiazepines (clobazam, diazepam and their main active metabolites) in human plasma by column-switching high-performance liquid chromatography, *J. Chromatogr.*, **1993**, 617, 285-290.

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## SAMPLE

**Matrix:** blood

**Sample preparation:** 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100  $\mu\text{L}$  mobile phase, centrifuge at 2800 g for 5 min, inject a 50  $\mu\text{L}$  aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

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## HPLC VARIABLES

**Column:** 300  $\times$  3.9  $\mu\text{m}$  NovaPack C18

**Mobile phase:** MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic))  $\text{KH}_2\text{PO}_4$  adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

**Column temperature:** 30

**Flow rate:** 0.8

**Injection volume:** 50

**Detector:** UV 229

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## CHROMATOGRAM

**Retention time:** 8.29

**Limit of detection:** <120 ng/mL

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## KEY WORDS

whole blood; plasma; interferences may occur—compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfapyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephensin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine;



prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vandesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phenacyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorphenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluoperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tiocloamarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

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## REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

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## SAMPLE

**Matrix:** blood, CSF

**Sample preparation:** 200  $\mu$ L Serum, plasma, or CSF + 300  $\mu$ L reagent. Flush column A to waste with 500  $\mu$ L 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500  $\mu$ L 500 mM ammonium sulfate, elute the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine hydrochloride and 1.02 M ammonium sulfate in water.)

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## HPLC VARIABLES

**Column:** A 30  $\times$  2.1 40  $\mu$ m preparative grade C18 (Analytichem); B 250  $\times$  4.6 10  $\mu$ m Partisil C8

**Mobile phase:** Gradient. A was 50 mM pH 4.5  $\text{KH}_2\text{PO}_4$ . B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 15 min, maintain at 30:70 for 4 min.

**Column temperature:** 50

**Flow rate:** 1.5

**Detector:** UV 280 for 5 min then UV 254

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## CHROMATOGRAM

**Retention time:** 17.73

**Internal standard:** heptanophenone (19.2)

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## OTHER SUBSTANCES

**Extracted:** acetazolamide, ampicillin, bromazepam, caffeine, carbamazepine, chloramphenicol, chlorothiazide, diazepam, droperidol, ethionamide, furosemide, isoniazid, methadone, penicillin G, phenobarbital, phenytoin, propoxyphene, pyrazinamide, rifampin, trimeprazine, trimethoprim

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## KEY WORDS

plasma; serum; column-switching

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## REFERENCE

Seifart,H.I.; Kruger,P.B.; Parkin,D.P.; van Jaarsveld,P.P.; Donald,P.R. Therapeutic monitoring of antituberculosis drugs by direct in-line extraction on a high-performance liquid chromatography system, *J.Chromatogr.*, **1993**, *619*, 285–290.

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**SAMPLE**

**Matrix:** blood, CSF, gastric contents, urine

**Sample preparation:** 200  $\mu$ L Serum, urine, CSF, or gastric fluid + 300  $\mu$ L reagent. Flush column A to waste with 500  $\mu$ L 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500  $\mu$ L 500 mM ammonium sulfate, backflush the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine HCl and 1.02 M ammonium sulfate in water.)

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**HPLC VARIABLES**

**Column:** A 40  $\mu$ m preparative grade C18 (Analytichem); B 75  $\times$  2.1 pellicular C18 (Whatman) + 250  $\times$  4.6 5  $\mu$ m C8 end-capped (Whatman)

**Mobile phase:** Gradient. A was 50 mM pH 4.5  $\text{KH}_2\text{PO}_4$ . B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 20 min.

**Column temperature:** 50

**Flow rate:** 1.5

**Detector:** UV 220

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**CHROMATOGRAM**

**Retention time:** 16.92

**Internal standard:** heptanophenone (19)

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**OTHER SUBSTANCES**

**Extracted:** acetaminophen, allobarbitol, azinphos, barbitol, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, pentobarbital, propoxyphene, prothiophos, quinine, salicylic acid, secobarbital, strychnine, sulfamethoxazole, theophylline, thiopental, thioridazine, trimethoprim

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**KEY WORDS**

serum; column-switching

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**REFERENCE**

Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J. Chromatogr.*, **1993**, 612, 191–198.

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**SAMPLE**

**Matrix:** blood, tissue, urine

**Sample preparation:** 1 mL Blood, urine, or liver homogenate + 1 mL 1.15 M pH 6.4 phosphate buffer, add 25  $\mu$ g prazepam, extract with 5 mL n-butyl chloride, centrifuge. Remove the organic layer and evaporate it in a vortex-evaporator. Reconstitute the residue in 100  $\mu$ L MeOH, inject a 20  $\mu$ L aliquot.

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**HPLC VARIABLES**

**Column:** 250  $\times$  4.6 Partisil-10 ODS

**Mobile phase:** MeCN:1 mM pH 3.2 phosphate buffer 40:60

**Column temperature:** 50

**Flow rate:** 3

**Injection volume:** 20

**Detector:** UV 240

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**CHROMATOGRAM**

**Retention time:** 7.2

**Internal standard:** prazepam

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**OTHER SUBSTANCES**

**Simultaneous:** temazepam

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**KEY WORDS**

liver; prazepam is IS

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**REFERENCE**

Martin,C.D.; Chan,S.C. Distribution of temazepam in body fluids and tissues in lethal overdose, *J.Anal.Toxicol.*, **1986**, *10*, 77-78.

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**SAMPLE**

**Matrix:** blood, urine

**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

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**HPLC VARIABLES**

**Guard column:** 20 mm long Symmetry C18

**Column:** 250 × 4.6 5 µm Symmetry C8 (Waters)

**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

**Column temperature:** 30

**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

**Injection volume:** 10-30

**Detector:** UV 200.5

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**CHROMATOGRAM**

**Retention time:** 23.6

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**KEY WORDS**

whole blood

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**REFERENCE**

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149-163.

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**SAMPLE**

**Matrix:** microsomal incubations

**Sample preparation:** 2.5 mL Microsomal incubation + 2.5 mL acetone, add 30 µL diazepam in MeOH, add 2.5 mL chloroform, centrifuge. Remove the organic layer and evaporate it to dryness under reduced pressure at 40°, reconstitute the residue in 100 µL mobile phase, inject an aliquot.

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**HPLC VARIABLES**

**Column:** 250 × 6.2 7 µm Zorbax silica

**Mobile phase:** Hexane:EtOH:MeCN 95:3.33:1.67

**Flow rate:** 2

**Detector:** UV 232

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**CHROMATOGRAM**

**Retention time:** 12

**Internal standard:** diazepam (16)

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**OTHER SUBSTANCES**

**Extracted:** metabolites, oxazepam

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**KEY WORDS**

human; liver; normal phase; pharmacokinetics

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**REFERENCE**

Lu,X.-L.; Guengerich,F.P.; Yang,S.K. Stereoselective metabolism of prazepam and halazepam by human liver microsomes, *Drug Metab.Dispos.*, **1991**, 19, 637-642.

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**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Inject a 50  $\mu$ L aliquot of a solution in mobile phase.

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**HPLC VARIABLES**

**Column:** 250  $\times$  4 10  $\mu$ m LiChrosorb RP-18

**Mobile phase:** MeCN:buffer 45:55 (Buffer was 800 mL 62.5 mM sodium acetate adjusted to pH 5.0 with 1 M NaOH and made up to 1 L.)

**Flow rate:** 1.5

**Injection volume:** 50

**Detector:** UV 225

---

**CHROMATOGRAM**

**Retention time:** 14.5

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**OTHER SUBSTANCES**

**Simultaneous:** flurazepam, pentobarbital, cimetidine, clorazepate, diazepam, oxazepam, nordiazepam

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**REFERENCE**

Colin,P.; Sirois,G.; Leloirier,J. High-performance liquid chromatography determination of dipotassium clorazepate and its major metabolite nordiazepam in plasma, *J.Chromatogr.*, **1983**, 273, 367-377.

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**SAMPLE**

**Matrix:** solutions

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**HPLC VARIABLES**

**Guard column:** 30  $\times$  2.1 Spheri-5 RP-8

**Column:** 220  $\times$  2.1 Spheri-5 RP-8

**Mobile phase:** Gradient. A was 0.08% diethylamine and 0.09% phosphoric acid in water, pH 2.3. B was MeCN:water 90:10 containing 0.08% diethylamine and 0.09% phosphoric acid. A:B 95:5 for 2 min, to 0:100 over 15 min, maintain at 0:100 for 5 min.

**Column temperature:** 50

**Flow rate:** 0.5

**Detector:** UV 200

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**CHROMATOGRAM**

**Retention time:** 15

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**OTHER SUBSTANCES**

**Simultaneous:** chlordiazepoxide, desalkylflurazepam, diazepam, flurazepam, norchlordiazepoxide, nordiazepam, oxazepam

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**REFERENCE**

*Rainin Catalog 1991-2*, p. 3.26.

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**SAMPLE**

**Matrix:** solutions

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**HPLC VARIABLES**

**Guard column:** 30  $\times$  2.1 Spheri-5 RP-8

**Column:** 220  $\times$  2.1 Spheri-5 RP-8

**Mobile phase:** Gradient. A was 0.08% diethylamine and 0.09% phosphoric acid in water, pH 2.3. B was MeCN:water 90:10 containing 0.08% diethylamine and 0.09% phosphoric acid. A:B 95:5 for 2 min, to 0:100 over 15 min (?), maintain at 0:100 for 5 min.

**Column temperature:** 50

**Flow rate:** 0.5  
**Detector:** UV 200

## CHROMATOGRAM

**Retention time:** 15

## OTHER SUBSTANCES

**Simultaneous:** norchlordiazepoxide, chlordiazepoxide, nordiazepam, desalkylflurazepam, oxazepam, diazepam, flurazepam

**Also analyzed:** amitriptyline, amphetamine, chlorpromazine, desipramine, desmethyldoxepin, diethylpropion, doxepin, ephedrine, fenfluramine, imipramine, mesoridazine, methamphetamine, nortriptyline, phentermine, phenylpropanolamine, promazine, thioridazine, thiothixene, trifluoperazine

## REFERENCE

*Rainin Catalog, C1-94, 1994, p. 7.24.*

## SAMPLE

**Matrix:** solutions

## HPLC VARIABLES

**Column:** 250 × 4.6 Zorbax RX

**Mobile phase:** Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

**Column temperature:** 30

**Flow rate:** 2

**Detector:** UV 210

## OTHER SUBSTANCES

**Also analyzed:** acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepam, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fen-camfamine, fenpropofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, imino-stilbene, imipramine, indomethacin, isocarboxystyl, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephénytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methypylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nyldrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phenacyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, prednisolone, primidone, proben-

acid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyridylidone, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sulfadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, translycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleminamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

## REFERENCE

Hill, D.W.; Kind, A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J. Anal. Toxicol.*, **1994**, *18*, 233-242.

## SAMPLE

**Matrix:** solutions

**Sample preparation:** Inject a 10  $\mu$ L aliquot.

## HPLC VARIABLES

**Column:** 250  $\times$  4.6 Vydac C18

**Mobile phase:** MeCN:20 mM pH 7.0 phosphate buffer 55:45

**Injection volume:** 10

**Detector:** UV 254

## CHROMATOGRAM

**Retention time:** 7

## OTHER SUBSTANCES

**Simultaneous:** temazepam

## REFERENCE

Yang, S.K. Acid-catalyzed ethanolysis of temazepam in anhydrous and aqueous ethanol solutions, *J. Pharm. Sci.*, **1994**, *83*, 898-902.

## SAMPLE

**Matrix:** solutions

## HPLC VARIABLES

**Column:** 250  $\times$  4.6 5  $\mu$ m Supelcosil LC-DP (A) or 250  $\times$  4.5  $\mu$ m LiChrospher 100 RP-8 (B)

**Mobile phase:** MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)

**Flow rate:** 0.6

**Injection volume:** 25

**Detector:** UV 229

## CHROMATOGRAM

**Retention time:** 10.49 (A), 12.77 (B)

## OTHER SUBSTANCES

**Also analyzed:** acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordi-azepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyrindamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide,

ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazinol, mefenamic acid, meperidine, mephénytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyl dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

## KEY WORDS

details of plasma extraction

## REFERENCE

Koves, E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J. Chromatogr. A*, **1995**, 692, 103–119.

## SAMPLE

**Matrix:** urine

**Sample preparation:** 1 mL Urine + 100  $\mu$ L 5 mM pH 5.5 acetate buffer + 25  $\mu$ L  $\beta$ -glucuronidase/arylsulfatase (0.235/0.065 U, Calbiochem), mix, heat at 37° for 16 h, add 50  $\mu$ L MeOH, add 1 mL saturated trisodium phosphate, add 3 mL dichloromethane, vortex for 2 min, centrifuge at 1610 g for 5 min. Remove a 2 mL aliquot of the organic layer and add it to 2 mL hexane and 2 mL 6 M HCl, vortex for 2 min, centrifuge at 1610 g for 5 min. Remove 1 mL of the aqueous phase and adjust pH to 6 with 1 mL 6 M NaOH and 1 mL saturated trisodium phosphate, add 3 mL dichloromethane, vortex for 2 min, centrifuge at 1610 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 150  $\mu$ L mobile phase, inject a 60  $\mu$ L aliquot.

## HPLC VARIABLES

**Column:** 250  $\times$  4.5  $\mu$ m LiChrospher 100 RP-18(e)

**Mobile phase:** MeOH:water:triethylamine 30:70:0.1 adjusted to pH 5.5 with phosphoric acid

**Flow rate:** 0.7

**Injection volume:** 60

**Detector:** UV 240

## CHROMATOGRAM

**Retention time:** 17.0

**Internal standard:** prazepam

## OTHER SUBSTANCES

**Extracted:** desmethyldiazepam, diazepam, oxazepam, temazepam

**Simultaneous:** amitriptyline, caffeine, carbamazepine, chlordiazepoxide, chlorpromazine, clonazepam, desipramine, flunitrazepam, flurazepam, haloperidol, imipramine, levomepromazine, maprotiline, mianserin, nitrazepam, nortriptyline, perphenazine, phenobarbital, phenytoin, sulpride, thioridazine, triazolam

## KEY WORDS

prazepam is IS

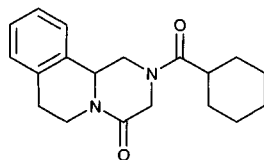
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**REFERENCE**

Chiba,K.; Horii,H.; Chiba,T.; Kato,Y.; Hirano,T.; Ishizaki,T. Development and preliminary application of high-performance liquid chromatographic assay of urinary metabolites of diazepam in humans, *J.Chromatogr.B*, **1995**, 668, 77–84.

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# Praziquantel



**Molecular formula:**  $C_{19}H_{24}N_2O_2$

**Molecular weight:** 312.41

**CAS Registry No.:** 55268-74-1

**Merck Index:** 7896

**Lednicer No.:** 4 213

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** Acidify 1 mL plasma with 300  $\mu$ L 1 M phosphoric acid, add 4 mL toluene, shake mechanically for 20 min, centrifuge at 1800 g, remove 3.5 mL of the organic layer. Add 1 mL water and 200  $\mu$ L 1.5 M NaOH, shake in a mixer for 1 min, centrifuge. Remove a 3 mL aliquot of the organic layer and evaporate it under a stream of air. Dissolve the residue in 100  $\mu$ L mobile phase, inject a 50  $\mu$ L aliquot.

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**HPLC VARIABLES**

**Guard column:** 4  $\times$  4 CN (Merck)

**Column:** 150  $\times$  4.6 5  $\mu$ m Chiralcel OD-H (Daicel, Chiral Technology)

**Mobile phase:** Hexane:EtOH 85:15

**Flow rate:** 1

**Injection volume:** 50

**Detector:** UV 220

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**CHROMATOGRAM**

**Retention time:** 9 (R-(-)), 12 (S-(+))

**Limit of quantitation:** 5 ng/mL (R-(-)), 10 ng/mL (S-(+))

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**OTHER SUBSTANCES**

**Interfering:** albendazole sulfone, albendazole metabolite, carbamazepine, clonazepam, lorazepam, triazolam

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**KEY WORDS**

chiral

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**REFERENCE**

Jabor,V.A.P.; Rocha,G.M.; Bonato,P.S. Enantioselective analysis of praziquantel in plasma samples, *J.Chromatogr.B*, **1997**, 696, 307–311.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** Condition a 100 mg Sep-Pak Vac C18 SPE cartridge with 3 mL MeOH, 1 mL water, and 5 mL 100 mM sodium carbonate. Mix 1.5 mL heparinized plasma with 1.5 mL 100 mM sodium carbonate, add to the SPE cartridge, wash with 5 mL 100 mM sodium carbonate, dry the cartridge in air for 30 s, elute with 750  $\mu$ L MeCN:0.2% phosphoric acid 50:50, vortex the eluate (protect from light), inject an aliquot.

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**HPLC VARIABLES**

**Guard column:** 20  $\times$  4.6 5  $\mu$ m BST Nucleosil C18

**Column:** 150  $\times$  3.9 4  $\mu$ m Novapak C18

**Mobile phase:** MeCN:buffer 33:67 (Buffer was 50 mM  $KH_2PO_4$  adjusted to pH 3.0 with 20% phosphoric acid.)



**Flow rate:** 1.5  
**Injection volume:** 50  
**Detector:** UV 220

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#### CHROMATOGRAM

**Retention time:** 8.2  
**Limit of detection:** 4 ng/mL  
**Limit of quantitation:** 25 ng/mL

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#### OTHER SUBSTANCES

**Extracted:** fenbendazole

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#### KEY WORDS

dog; pharmacokinetics; plasma; SPE

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#### REFERENCE

Morovján,G.; Csokán,P.; Makranaszki,L.; Abdellah-Nagy,E.A.; Tóth,K. Determination of fenbendazole, praziquantel and pyrantel pamoate in dog plasma by high-performance liquid chromatography, *J.Chromatogr.A*, 1998, 797, 237–244.

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#### SAMPLE

**Matrix:** blood, microsomal incubations

**Sample preparation:** Extract 1 mL serum or microsomal incubation three times with 2 mL ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of air at 60°, reconstitute the residue in 200 µL mobile phase, inject a 50 µL aliquot.

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#### HPLC VARIABLES

**Guard column:** 40 × 4 15 µm RP-18

**Column:** 200 × 4 5 µm RP-18

**Mobile phase:** MeCN:water 50:50

**Flow rate:** 1.5

**Injection volume:** 50

**Detector:** UV 210

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#### KEY WORDS

serum; rat; human

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#### REFERENCE

Masimirembwa,C.M.; Naik,Y.S.; Hasler,J.A. The effect of chloroquine on the pharmacokinetics and metabolism of praziquantel in rats and in humans, *Biopharm.Drug Dispos.*, 1994, 15, 33–43.

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#### SAMPLE

**Matrix:** blood, tissue

**Sample preparation:** Plasma. 250 µL Plasma + 100 µL water + 1 mL acetone, homogenize for 10 s, add 50 µL 1 M NaOH, add 2 mL hexane:diethyl ether 40:60, mix for 10 s, centrifuge at 3000 rpm for 3 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 200 µL MeOH:buffer 70:30, add 500 µL hexane, mix, centrifuge for 3 min, discard the hexane layer. Remove 100 µL of the MeOH layer and add it to 100 µL 10 mM phosphoric acid, mix, filter (Costar Spin-X 0.2 µm nylon) while centrifuging at 5600 g for 4 min, inject a 25 µL aliquot of the filtrate. Muscle. 3 g Muscle + 300 µL water + 4.7 mL acetone, homogenize (Ultra-Turrax TP 18/2) for 6 s, centrifuge at 5000 rpm for 3 min. Remove 4 mL of the supernatant and add it to 5 mL hexane:diethyl ether 40:60, shake vigorously for 10 s, centrifuge at 3000 rpm for 3 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 400 µL MeOH:buffer 70:30, let stand at -20° for 5 min, centrifuge at 3000 rpm for 3 min. Remove 300 µL of the MeOH based phase and add it to 300 µL water, filter (Costar Spin-X 0.2 µm nylon) while centrifuging, inject a 25 µL aliquot of the filtrate. Liver. 3 g Liver + 300 µL water + 4.7 mL acetone, homogenize (Ultra-Turrax TP 18/2) for 6 s, centrifuge at 5000 rpm for 3 min. Remove 4 mL of the supernatant and add it to 5 mL hexane:diethyl ether 40:60, shake vigorously for 10 s, centrifuge at 3000 rpm for 3 min. Remove the organic layer and add it to 50 µL 1 M NaOH, shake vigorously for 5 s, centrifuge for 2 min. Remove the upper layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 600 µL MeOH:

buffer 70:30, add 1 mL hexane, mix, centrifuge for 3 min. Discard the hexane layer and add 240  $\mu$ L of the MeOH based layer to 160  $\mu$ L 20 mM phosphoric acid, mix for 3 s, filter (Costar Spin-X 0.2  $\mu$ m nylon) while centrifuging, inject a 25  $\mu$ L aliquot of the filtrate. (Buffer was 4.45 g sodium heptanesulfonate and 1.779 g  $\text{Na}_2\text{HPO}_4$  in 750 mL water, adjust pH to 6 with 2 M phosphoric acid, make up to 1 L with water.)

**HPLC VARIABLES**

**Guard column:** 20  $\times$  4.6 5  $\mu$ m Supelcosil LC-ABZ

**Column:** 150  $\times$  4.6 5  $\mu$ m Supelcosil LC-ABZ

**Mobile phase:** MeCN:water 40:60 (muscle) or 39:61 (liver, plasma) (After each injection wash with MeCN for 5 min at 1.5 mL/min, wash with mobile phase for 7 min at 1.8 mL/min and for 3 min at 1 mL/min.)

**Flow rate:** 1 for 3 min then 0.8 for 6 min

**Injection volume:** 25

**Detector:** UV 205

**CHROMATOGRAM**

**Retention time:** 8.5

**Limit of quantitation:** 15 ng/g (liver), 20 ng/mL (plasma), 5 ng/g (muscle)

**KEY WORDS**

plasma; fish; trout; muscle; liver

**REFERENCE**

Hormazal,V.; Yndestad,M. High-performance liquid chromatographic determination of praziquantel in plasma and tissues of cultured fish for residue and pharmacokinetic studies, *J.Liq.Chromatogr.*, **1995**, *18*, 589–597.

**SAMPLE**

**Matrix:** blood, tissue, urine

**Sample preparation:** Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 5 mL 50 mM pH 5.0 phosphate buffer. 2 mL plasma, urine, or 10% liver homogenate + 100  $\mu$ L 10  $\mu$ g/mL IS + 1 mL 200 mM NaOH, vortex for 15 s, add to the SPE cartridge, wash with 20 mL 50 mM pH 5.0 phosphate buffer, wash with 8 mL MeOH, elute with two 3 mL aliquots of ethyl acetate:diisopropyl ether 70:30 (plasma, urine) or 30:70 (liver) (Caution! Diisopropyl ether readily forms explosive peroxides!). Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100  $\mu$ L mobile phase, inject a 20  $\mu$ L aliquot.

**HPLC VARIABLES**

**Column:** 250  $\times$  4.6 Ultrasphere ODS C18

**Mobile phase:** MeCN:water 45:55 (plasma, urine) or 50:50 (liver)

**Flow rate:** 1.5

**Injection volume:** 20

**Detector:** UV 217

**CHROMATOGRAM**

**Retention time:** 6.1 (plasma, urine), 4.9 (liver)

**Internal standard:** 2-cycloheptylcarbonyl-4-oxo-1,2,3,6,7,11b-hexahydro-4H-pyrzino[2,1-a]isoquinoline (8.7 (plasma, urine), 6.5 (liver))

**Limit of detection:** 31.2 ng/mL

**KEY WORDS**

SPE; plasma; liver; human; rat

**REFERENCE**

González-Esquivel,D.F.; Okuno,C.M.; Sánchez Rodríguez,M.; Sotelo Morales,J.; Cook,H.J. Sensitive high-performance liquid chromatographic assay for praziquantel in plasma, urine and liver homogenates, *J.Chromatogr.*, **1993**, *613*, 174–178.

**SAMPLE**

**Matrix:** feed, sediment

**Sample preparation:** Feed. 0.5 g Ground feed + 6 mL acetone, mix for 5 s, let stand for 5 min, whirlmix for 5 s, make up to 50 mL with MeCN:water 40:60, blend, centrifuge at 3000 rpm for 3 min. Remove 0.5 mL of the supernatant and add it to 4.5 mL MeCN:water 40:60, blend, filter (Costar 0.2  $\mu$ m nylon membrane Spin-X centrifuge filter) while centrifuging at 5600 g for 3 min, inject a 10  $\mu$ L aliquot of the filtrate. Sediment. 2 g Sediment + 200  $\mu$ L water + 6 mL acetone, mix for 5 s, let stand for 5 min, whirlmix for 5 s, centrifuge at 5000 rpm for 3 min. 4.1 mL Supernatant + 50  $\mu$ L 1 M NaOH + 5 mL diethyl ether:hexane 60:40, shake vigorously for 5 s, centrifuge at 3000 rpm for 3 min. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 1 mL MeOH:buffer 70:30 and 1 mL hexane, whirlmix, centrifuge for 3 min, discard the hexane layer, add 2 mL MeCN:10 mM phosphoric acid 40:60, mix, filter a 500  $\mu$ L aliquot (Costar 0.2  $\mu$ m nylon membrane Spin-X centrifuge filter) while centrifuging at 5600 g for 3 min, inject a 20  $\mu$ L aliquot of the filtrate. (Buffer was 4.45 g sodium 1-heptanesulfonate and 1.779 g NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O in 750 mL water, adjust pH to 6 with 2 M phosphoric acid, make up to 1 L with water.)

#### HPLC VARIABLES

**Guard column:** 20  $\times$  4.6 5  $\mu$ m Supelcosil ABZ

**Column:** 150  $\times$  4.6 5  $\mu$ m Supelcosil ABZ

**Mobile phase:** MeCN:water 40:60 (feed) or 39:61 (sediment)

**Flow rate:** 1 (feed) or 1 for 3 min, then 0.8 for 6 min (sediment)

**Injection volume:** 10-20

**Detector:** UV 205

#### CHROMATOGRAM

**Retention time:** 7 (feed), 9 (sediment)

**Limit of quantitation:** 500 ng/g (feed), 30 ng/g (sediment)

#### REFERENCE

Hormazal,V.; Yndestad,M. Determination of praziquantel in medicated fish feed and sediment by HPLC, *J.Liq.Chromatogr.*, **1995**, 18, 1231-1238.

## Prazosin

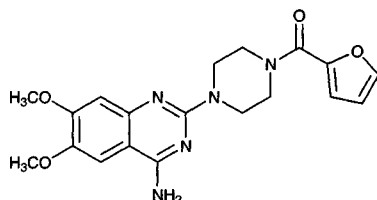
**Molecular formula:** C<sub>19</sub>H<sub>21</sub>N<sub>5</sub>O<sub>4</sub>

**Molecular weight:** 383.41

**CAS Registry No.:** 19216-56-9, 19237-84-4 (HCl)

**Merck Index:** 7897

**Lednicer No.:** 2 382



#### SAMPLE

**Matrix:** blood

**Sample preparation:** 1 mL Plasma + 100  $\mu$ L 2 M NaOH, extract with 5 mL pentane:dichloromethane 2:1. Remove the organic layer and evaporate it to dryness under a gentle stream of nitrogen, reconstitute the residue in mobile phase, inject a 10  $\mu$ L aliquot.

#### HPLC VARIABLES

**Column:** 100  $\times$  2.1 10  $\mu$ m Chiralpak AD (Chiral Technologies, Exton, PA)

**Mobile phase:** n-Hexane:isopropanol:diethylamine 70:30:0.1

**Column temperature:** 30

**Flow rate:** 0.2

**Injection volume:** 10

**Detector:** MS, SCIEX API 300 tandem mass, positive ion mode, nebulizer 440°, scan 384.0/247.0

#### CHROMATOGRAM

**Retention time:** 4.50

**Internal standard:** prazosin

#### OTHER SUBSTANCES

**Extracted:** doxazosin

**KEY WORDS**

plasma; small-bore; prazosin is IS

**REFERENCE**

Alebic-Kolbah, T.; Zavitsanos, A. P. Chiral bioanalysis by normal high-performance liquid chromatography-atmospheric pressure ionization tandem mass spectrometry, *J.Chromatogr.B*, **1997**, 759, 65–77.

**SAMPLE**

**Matrix:** blood

**Sample preparation:** Condition a 100 mg Bond Elut C18 SPE cartridge with two 1 mL portions of MeOH and two 1 mL portions of 50 mM pH 7.2  $\text{KH}_2\text{PO}_4$  buffer. Centrifuge whole blood at 1500 g for 10 min. Add 1 mL 50 mM pH 7.2  $\text{KH}_2\text{PO}_4$  buffer to 1 mL plasma, vortex for 5 s, add to the SPE cartridge, dry in a stream of air, wash with two 1 mL portions of 50 mM pH 7.2  $\text{KH}_2\text{PO}_4$  buffer, wash with 500  $\mu\text{L}$  MeOH. Dry the cartridge in a stream of air, let stand for 15 min, elute with 1 mL MeCN:25% ammonium hydroxide 99:1, evaporate the eluate to dryness under a stream of nitrogen, dissolve the residue in 200  $\mu\text{L}$  mobile phase, inject an aliquot.

**HPLC VARIABLES**

**Guard column:** 30  $\times$  4.6 Supelguard ABZ+plus C18 (Supelco)

**Column:** 250  $\times$  4.6 Supelcosil ABZ+plus C18 (Supelco)

**Mobile phase:** MeCN:50 mM pH 6.5  $\text{KH}_2\text{PO}_4$  buffer 30:70

**Flow rate:** 1

**Detector:** E, ESA Coulochem II, 5011 model analytical cell, guard cell +950 mV, first electrode +600 mV, second electrode +900 mV

**CHROMATOGRAM**

**Retention time:** 6.14

**Internal standard:** prazosin

**OTHER SUBSTANCES**

**Extracted:** buspirone

**KEY WORDS**

plasma; prazosin is IS; SPE

**REFERENCE**

Ary,K.; Róna,K.; Ondi,S.; Gachályi,B. High-performance liquid chromatographic method with coulometric detection for the determination of buspirone in human plasma by means of a column-switching technique, *J.Chromatogr.A*, **1998**, 797, 221–226.

**SAMPLE**

**Matrix:** blood

**Sample preparation:** Add 500  $\mu\text{L}$  5 M NaOH to 1 mL plasma, add 5 mL ethyl acetate, extract. Centrifuge at 700 g for 10 min, evaporate 4 mL of the organic phase to dryness under nitrogen at 60°, reconstitute the residue in 200  $\mu\text{L}$  mobile phase, inject a 20  $\mu\text{L}$  aliquot.

**HPLC VARIABLES**

**Column:** 250  $\times$  4.6 5  $\mu\text{m}$  Nucleosil C18

**Mobile phase:** MeCN:buffer 69:31 (Mobile phase was 690 mL MeCN, 310 mL water, and 9 mL glacial acetic acid, adjusted to pH 5.0 with 5 M NaOH.)

**Flow rate:** 1.5

**Injection volume:** 20

**Detector:** F ex 362 em 414

**CHROMATOGRAM**

**Retention time:** 3.8

**Internal standard:** prazosin (3.8)

**OTHER SUBSTANCES**

**Extracted:** amiloride

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**KEY WORDS**

plasma; prazosin is IS

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**REFERENCE**

Jankowski,A.; Skorek-Jankowska,A.; Lamparczyk,H. Determination and pharmacokinetics of a furosemide-amiloride drug combination, *J.Chromatogr.B*, **1997**, 693, 383–391.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** Place 50  $\mu\text{L}$  of a 100  $\mu\text{g}/\text{mL}$  solution of doxazosin in MeOH into the bottom of a tube, evaporate to dryness under a stream of nitrogen at 37°, add 1 mL whole blood, mix thoroughly, add 5 mL diethyl ether, shake for 10 min, centrifuge at 2000 rpm for 5 min, freeze in acetone/dry ice. Remove the organic layer and add it to 100  $\mu\text{L}$  50 mM sulfuric acid, shake for 10 min, centrifuge at 2000 rpm for 5 min, inject a 20  $\mu\text{L}$  aliquot of the aqueous layer.

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**HPLC VARIABLES**

**Column:** 250  $\times$  4.5  $\mu\text{m}$  Spherisorb ODS

**Mobile phase:** MeOH:water 42:58 containing 10 mM pentane sodium sulfate and 11.6 mM tetramethylammonium chloride, adjusted to pH 3.4 with glacial acetic acid

**Flow rate:** 1.8

**Injection volume:** 20

**Detector:** F ex 254 em 400 (cut-off filter)

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**CHROMATOGRAM**

**Internal standard:** doxazosin

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**OTHER SUBSTANCES**

**Extracted:** trimazosin

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**KEY WORDS**

whole blood

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**REFERENCE**

Hughes,M.A.; Meredith,P.A.; Elliott,H.L. The determination of trimazosin and its metabolite CP23445 in whole blood by high performance liquid chromatography using fluorescence detection, *J.Pharmacol.Methods*, **1984**, 12, 29–34.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** 200  $\mu\text{L}$  Plasma + 200  $\mu\text{L}$  1 M NaOH + 12.5  $\mu\text{L}$  100  $\mu\text{g}/\text{mL}$  propyl hydroxybenzoate in MeOH + 7 mL diethyl ether, vortex for 1 min, centrifuge at 3000 rpm for 2 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 400  $\mu\text{L}$  mobile phase, inject an aliquot.

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**HPLC VARIABLES**

**Column:** 150  $\times$  4.6  $\mu\text{m}$  Adsorbosphere phenyl

**Mobile phase:** MeCN:50 mM phosphate buffer 30:70 adjusted to pH 3.3–3.4 with phosphoric acid

**Flow rate:** 1.5

**Detector:** F ex 247 em 394

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**CHROMATOGRAM**

**Retention time:** 4.0

**Internal standard:** propyl hydroxybenzoate (6.0)

**Limit of quantitation:** 0.5 ng/mL

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**KEY WORDS**

plasma

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**REFERENCE**

Niazzy,E.M.; El-Sayed,Y.M.; Khidr,S.H. Analysis of prazosin in plasma by high-performance liquid chromatography using fluorescence detection, *J.Liq.Chromatogr.*, **1995**, 18, 977–987.

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**SAMPLE****Matrix:** blood**Sample preparation:** 100  $\mu$ L Serum + 200  $\mu$ L MeCN, centrifuge at 9000 g for 5 min. Remove the supernatant and add it to 250  $\mu$ L water-saturated n-hexane, vortex for 2 min, centrifuge at 10000 rpm for 5 min, discard the hexane layer, repeat the hexane wash twice more. Evaporate the MeCN layer to dryness under a stream of nitrogen, reconstitute with 100  $\mu$ L mobile phase, inject a 50  $\mu$ L aliquot.

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**HPLC VARIABLES****Column:** 150  $\times$  4.6 ODS Hypersil**Mobile phase:** MeCN:50 mM Na<sub>2</sub>HPO<sub>4</sub> 40:60, pH 8.4**Flow rate:** 1**Injection volume:** 50**Detector:** E, ESA Coulochem II, No. 5014 analytical cell, +500 mV on channel 1 (monitoring), channel 2 +0.00 mV, guard cell +300 mV

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**CHROMATOGRAM****Retention time:** 5.7**Limit of detection:** 2.5 ng/mL

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**KEY WORDS**

serum; fetal; cow

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**REFERENCE**Rathinavelu,A.; Malave,A. High-performance liquid chromatography using electrochemical detection for the determination of prazosin in biological samples, *J.Chromatogr.B*, **1995**, 670, 177–182.

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**SAMPLE****Matrix:** blood**Sample preparation:** 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100  $\mu$ L mobile phase, centrifuge at 2800 g for 5 min, inject a 50  $\mu$ L aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

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**HPLC VARIABLES****Column:** 300  $\times$  3.9  $\mu$ m NovaPack C18**Mobile phase:** MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH<sub>2</sub>PO<sub>4</sub> adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)**Column temperature:** 30**Flow rate:** 0.8**Injection volume:** 50**Detector:** UV 247

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**CHROMATOGRAM****Retention time:** 3.89**Limit of detection:** <120 ng/mL

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**KEY WORDS**

whole blood; plasma; interferences may occur—compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lor-

azepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; videsine; mexiletine; dipyrindamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phenacyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loperazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluoperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thiopropazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tiocloamarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

## REFERENCE

Tracqui, A.; Kintz, P.; Mangin, P. Systematic toxicological analysis using HPLC/DAD, *J. Forensic Sci.*, **1995**, *40*, 254–262.

## SAMPLE

**Matrix:** blood, urine

**Sample preparation:** 50  $\mu$ L Plasma or urine + 100  $\mu$ L saturated NaCl + 50  $\mu$ L 2  $\mu$ g/mL dimethothiazine mesylate in water + 50  $\mu$ L 4 M NaOH, vortex for 10 s, add 200  $\mu$ L MTBE, vortex for 30 s, centrifuge at 9950 g for 2 min, inject a 110  $\mu$ L aliquot of the organic phase.

## HPLC VARIABLES

**Column:** 250  $\times$  5 5  $\mu$ m Spherisorb S5W

**Mobile phase:** MeOH:10 mM ammonium perchlorate adjusted to pH 6.7 with 1 mL/L methanolic NaOH (0.1 M)

**Flow rate:** 2

**Injection volume:** 110

**Detector:** F ex 370 em 370–700

## CHROMATOGRAM

**Retention time:** 3

**Internal standard:** dimethothiazine mesylate (5)

**Limit of detection:** 1000 ng/mL

## OTHER SUBSTANCES

**Simultaneous:** N-acetylprocainamide, ajmaline, chlorpromazine, desipramine, dipyrindamole, doxazosin, flecainide, flurazepam, gallopamil, imipramine, ketanserin, metoprolol, mexiletine, mianserin, nadolol, nitrazepam, orphenadrine, oxprenolol, penbutolol, pindolol, prajmalium, procainamide, propranolol, protriptyline, pyrimethamine, quinidine, quinine, terazosin, triamterene, trimipramine, verapamil

**Noninterfering:** amiodarone, atenolol, disopyramide, labetalol, lignocaine, lorcanide, methyl-dopa, nifedipine, prenatalerol, propafenone, sotalol, timolol

## KEY WORDS

plasma

## REFERENCE

Bhamra, R.K.; Flanagan, R.J.; Holt, D.W. High-performance liquid chromatographic measurement of prazosin and terazosin in biological fluids, *J. Chromatogr.*, **1986**, *380*, 216–221.

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**SAMPLE**

**Matrix:** blood, urine

**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

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**HPLC VARIABLES**

**Guard column:** 20 mm long Symmetry C18

**Column:** 250 × 4.6 5 µm Symmetry C8 (Waters)

**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

**Column temperature:** 30

**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

**Injection volume:** 10-30

**Detector:** UV 246.4

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**CHROMATOGRAM**

**Retention time:** 10.608

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**KEY WORDS**

whole blood

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**REFERENCE**

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, 763, 149-163.

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**SAMPLE**

**Matrix:** perfusate

**Sample preparation:** Condition a Sep-Pak C18 SPE cartridge with 10 mL MeOH and 10 mL water. 1 mL Perfusate + 1 µg propranolol + 2 mL water, add to the SPE cartridge, wash with 10 mL water, dry under vacuum, elute with 5 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 µL mobile phase, centrifuge at 700 g for 5 min, inject a 30 µL aliquot.

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**HPLC VARIABLES**

**Column:** 250 × 4.6 5 µm Alltima C18 (Alltech)

**Mobile phase:** MeOH:50 mM pH 5.8 phosphate buffer 55:45, final pH 5.1

**Column temperature:** 40

**Flow rate:** 1

**Injection volume:** 30

**Detector:** F ex 280 em 395

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**CHROMATOGRAM**

**Retention time:** 4.9

**Internal standard:** propranolol (7.1)

**Limit of detection:** 0.1 ng/mL

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**OTHER SUBSTANCES**

**Noninterfering:** albuterol, alcuronium, aminophylline, atenolol, atropine, betamethasone, bupivacaine, cortisone, dexamethasone, diazepam, diltiazem, hydrocortisone, hyoscine, hyoscine-N-butylbromide, labetalol, lidocaine, methimazole, metoclopramide, norepinephrine, phenobarbital, L-phenylephrine, phenytoin, prednisolone, prednisone, promethazine, propylthiouracil, pyridoxine, ranitidine, verapamil



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**KEY WORDS****SPE**

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**REFERENCE**

Fletcher,A.J.; Addison,R.S.; Mortimer,R.H.; Cannell,G.R. Rapid determination of prazosin in perfusion media by HPLC with solid phase extraction, *J.Liq.Chromatogr.*, **1995**, 18, 2911-2923.

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**SAMPLE****Matrix:** solutions**Sample preparation:** Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.

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**HPLC VARIABLES****Column:** 125 × 4.9 Spherisorb S5W silica**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7**Flow rate:** 2**Injection volume:** 20**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

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**CHROMATOGRAM****Retention time:** 1.6

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**OTHER SUBSTANCES**

**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclozine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclimine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipamnone, diprenorphine, dipyrindamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, flupromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserine, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclorphenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazine, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenylglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozone, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenylidamine, theophylline, thiethylperazine, thiopropazate, thiopropazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranlycypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleminamine, triprolidine, tryptamine, verapamil, xylometazoline

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**REFERENCE**

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, 323, 191–225.

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**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Prepare a 50 µg/mL solution in MeCN:water 40:60, inject an aliquot.

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**HPLC VARIABLES**

**Column:** 250 × 4.6 3 µm silica (Phenomenex)

**Mobile phase:** MeCN:6.25 mM pH 3.0 phosphate buffer 40:60

**Flow rate:** 1

**Injection volume:** 50

**Detector:** UV 254

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**CHROMATOGRAM**

**Retention time:** 9.31

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**OTHER SUBSTANCES**

**Also analyzed:** atenolol, clonidine, diltiazem, metoprolol, nifedipine, propranolol, verapamil

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**REFERENCE**

Simmons,B.R.; Stewart,J.T. HPLC separation of selected cardiovascular agents on underivatized silica using an aqueous organic mobile phase, *J.Liq.Chromatogr.*, **1994**, 17, 2675–2690.

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**SAMPLE**

**Matrix:** solutions

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**HPLC VARIABLES**

**Column:** 150 × 4.6 12 µm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)

**Mobile phase:** MeCN:100 mM pH 7.0 phosphate buffer 20:80

**Flow rate:** 1

**Detector:** UV 254

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**CHROMATOGRAM**

**Retention time:** k' 39.26

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**OTHER SUBSTANCES**

**Also analyzed:** acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripeleppamine, triprolidine, tymazoline, UK-14,304

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**REFERENCE**

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α<sub>1</sub>-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, 9, 211–215.

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**SAMPLE**

**Matrix:** solutions

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**HPLC VARIABLES**

**Column:** 250 × 4.6 5 µm Supelcosil LC-DP (A) or 250 × 4.5 µm LiChrospher 100 RP-8 (B)

**Mobile phase:** MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)

**Flow rate:** 0.6  
**Injection volume:** 25  
**Detector:** UV 229

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**CHROMATOGRAM**

**Retention time:** 7.38 (A), 4.21 (B)

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**OTHER SUBSTANCES**

**Also analyzed:** acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyrindamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenopropfen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazinol, mefenamic acid, meperidine, mephentermine, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyl dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phenolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimizide, pindolol, piroxicam, pramoxine, prazepam, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

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**KEY WORDS**

details of plasma extraction

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**REFERENCE**

Koves, E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J. Chromatogr. A*, **1995**, 692, 103–119.

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**SAMPLE**

**Matrix:** tissue

**Sample preparation:** 300 mg Skin + 100  $\mu$ L 10  $\mu$ g/mL verapamil hydrochloride in PBS, let stand for 2 h, cut skin into small pieces, add 5 mL MeOH, homogenize, filter, homogenize residue again with MeOH four more times. Combine filtrates, evaporate to dryness, reconstitute in 1 mL mobile phase, inject a 10  $\mu$ L aliquot.

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**HPLC VARIABLES**

**Column:** 150  $\times$  4.6 cyano (Alltech)

**Mobile phase:** MeCN:MeOH:water 45:5:50 containing 3 mM sodium heptanesulfonate

**Flow rate:** 1.5

**Injection volume:** 10

**Detector:** UV 230

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**CHROMATOGRAM**

**Retention time:** 4.4

**Internal standard:** verapamil (5.8)

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**Limit of detection:** 50 ng/mL

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**KEY WORDS**

skin

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**REFERENCE**

Tenjarla,S.N.; Tseggai,A. High-performance liquid chromatographic assay of prazosin for transdermal screening studies, *J.Clin.Pharm.Ther.*, **1992**, 17, 37–42.

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# Prednicarbate

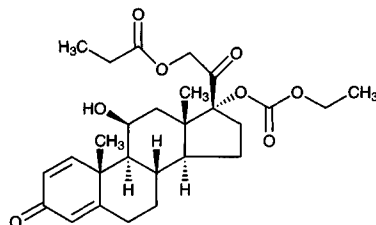
**Molecular formula:** C<sub>27</sub>H<sub>36</sub>O<sub>8</sub>

**Molecular weight:** 488.58

**CAS Registry No.:** 73771-04-7

**Merck Index:** 7899

**Lednicer No.:** 4 71



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**SAMPLE**

**Matrix:** tissue

**Sample preparation:** Extract incubation suspension twice with 3 mL portions of ethyl acetate, vortex for 1 min, centrifuge at 1000 rpm for 5 min, dry the combined organic phases under nitrogen, reconstitute with 1 mL MeOH, vortex 1 min, dry in a conical tube, reconstitute with 100 µL MeOH, centrifuge, inject a 20 µL aliquot.

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**HPLC VARIABLES**

**Column:** 125 × 4 5 µm LiChrospher 100 RP-18

**Mobile phase:** Gradient. A was MeCN. B was water. A:B from 20:80 to 100:0 over 20 min

**Flow rate:** 1

**Injection volume:** 20

**Detector:** UV 254

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**CHROMATOGRAM**

**Retention time:** 15.1

**Internal standard:** betamethasone (9.4)

**Limit of detection:** 10 ng/mL

**Limit of quantitation:** 100 ng/mL-50 µg/mL

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**OTHER SUBSTANCES**

**Extracted:** metabolites

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**KEY WORDS**

pharmacokinetics; keratinocytes; fibroblasts

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**REFERENCE**

Gysler,A.; Lange,K.; Korting,H.C.; Schäfer-Korting,M. Prednicarbate biotransformation in human foreskin keratinocytes and fibroblasts, *Pharm.Res.*, **1997**, 14, 793–797.